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Intrinsic features of the CD8α⁻ dendritic cell subset in inducing functional T follicular helper cells

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ABSTRACT

T follicular helper (Tfh) cells, a true B cell helper, have a critical role in enhancing humoral immune responses. However, the initial differentiation of Tfh cells by dendritic cells (DCs), the most potent antigen presenting cells, has not been clearly understood, particularly in the knowledge of the two major conventional dendritic cell subsets, CD8α⁺ DCs or CD8α⁻ DCs. Here we demonstrated that the localization of CD8α⁻ DCs in the marginal zone (MZ) bridging channels is closely associated with the induction of CXCR5⁺CCR7^{low} Tfh cells. We also showed that the major source of IL-6 for inducing Tfh cells is provided from the activated CD4⁺ T cells induced by CD8α⁻ DCs, and IL-6 directly secreted from the DC subsets seems minor. CD8α⁻ DCs were superior in inducing functional Tfh cells over other antigen presenting cells including B cells. We here observed the unknown intrinsic features of the DC subsets, suggesting the potential of utilizing the CD8α⁻ DC subset as therapeutic vaccine for the regulation of humoral immune responses.

1. Introduction

T follicular helper (Tfh) cells are identified as the true B cell helper and have been noted for their capability of modulating T cell dependent humoral immune responses [1,2]. Since the role of CXCR5 in Tfh cells was reported, leading its CXCL13 dependent migration into the germinal center (GC) [3,4], several discrete features of Tfh cells such as high expressions of ICOS and PD1 or low expression of CCR7, transcription factor Bcl6, and major cytokine IL-21 were identified [5]. In addition, the clinical importance of Tfh cells involved in humoral immunity related diseases such as autoimmunity and humoral immunodeficiency was reported [6], and thus the understanding the initiation of the Tfh cell differentiation is urgent and crucial. Although many researches have actively identified specific features of Tfh cells, how naïve CD4⁺ T cells differentiate into Tfh cells is not clearly understood.

Dendritic cell (DC), the most potent antigen presenting cell (APC), is the key modulator in inducing several CD4⁺ effector T cell subsets [7]. Two major conventional DC subsets in the secondary lymphoid organs are defined by the expression of CD8α, either CD11c⁺CD8α⁺ DCs expressing the endocytic receptor DEC-205 or CD11c⁺CD8⁻ DCs expressing the endocytic receptor DCIR2 [8,9]. We recently reported that the conventional CD8α⁻ DC subset is superior over CD8α⁺ DCs in inducing functional Tfh cells both *in vitro* and *in vivo*, generating efficient antigen specific humoral immune responses [10]. CD8α⁻ DCs were very effective in enhancing the number of antigen specific Tfh cells, the formation of germinal centers, and the quality and quantity of antibody titers against human pathogenic antigens such as *Yersinia pestis* LcrV, HIV Gag and Hepatitis B surface antigen [10]. We also reported the significance of ICOSL and OX40L expressed on CD8α⁻ DCs via the highly enhanced non-canonical NF-κB signaling pathway in inducing functional Tfh cells [10]. However, several important features such as the proximal location of the two DC subsets to antigen specific CD4⁺ T cells *in vivo*, cytokines secreted from the DC subsets and the potential involvement of other APCs, majorly B cells, in the induction of Tfh cells have not been addressed. Thus, the aim of the present study was to examine the undescribed intrinsic features of

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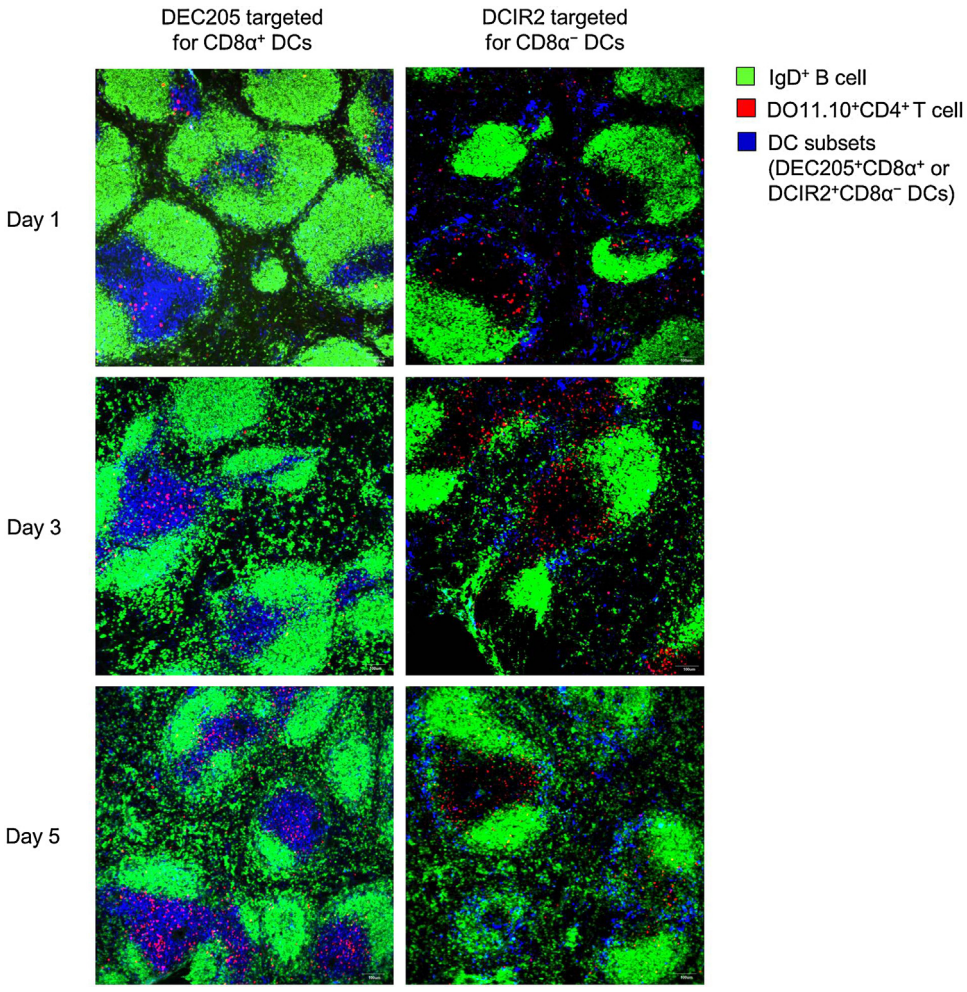

Fig. 1. $CD4^+$ T cells primed by $CD8\alpha^-$ DCs localized in the MZ bridging channels. OVA specific DO11.10$^+$CD4$^+$ T cells were adoptively transferred to naive BALB/c mice at day −1, and were immunized subcutaneously (s.c.) either with αDEC:OVA ($CD8\alpha^+$ DC targeting) or αDCIR2:OVA ($CD8\alpha^-$ DC targeting) conjugated monoclonal antibodies (mAbs) in the presence of poly (I:C) at day 0. At each indicated time point after the immunization, immunohistochemical staining of spleen sections from each group was prepared and detected by fluorescence confocal microscopy. IgD, green; DO11.10, red; DEC205 (for $CD8\alpha^+$ DCs) or DCIR2 (for $CD8\alpha^-$ DCs), blue. Data are representative of three or more independent experiments ($n > 10$ per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the two DC subsets in the initiation of Tfh cell differentiation by utilizing DC subset targeting strategy and purified DC subsets.

2. Materials and methods

2.1. Mice

BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased from Taconic, and OT-II and DO11.10 Thy 1.1 mice were purchased from The Jackson Laboratory. All mice were maintained under specific pathogen-free conditions and used at 6–8 weeks approved by Ulsan National Institute of Science and Technology Institutional Animal Care and Use Committee (approval number: UNISTIACUC-12-006-A).

2.2. Dendritic cell preparation

Total number of splenic dendritic cells was increased by Fms-like tyrosine 3 ligand (Flt3L) as previously described [9]. In brief, 5×10^6 cells of Flt3L-melanoma cells were subcutaneously (s.c.) injected to naïve C57BL/6 mice. After 10–14 days, the expanded splenic $CD11c^+$ DCs were enriched with positive magnetic-activated cell sorting (MACS; Miltenyi Biotec, GmbH, Bergisch Gladbach, Germany) and were further sorted into the two DC subsets, $CD3^-B220^-CD11c^+CD8\alpha^+$ or $CD3^-B220^-CD11c^+CD8\alpha^-$ DCs by MoFlo XDP (Beckman Coulter, Brea, CA, USA). To analyze cytokines or surface marker expressions in/on the two DC subsets, the purified each DC subset was stimulated either with 25 μg/ml poly (I:C) or 100 ng/ml LPS for 12 or 24 h *in vitro*.

2.3. $CD4^+$ T cell preparation

OVA-specific transgenic $CD4^+$ T cells from the lymph nodes and the spleen of OT-II or DO11.10 Thy1.1 mice were negatively isolated using hybridoma supernatant cocktail of rat-anti mouse −CD8 (2.43), −MHC class II (T1B120), −Mϕ (F4/80), −B220 (RA3-6B2), and −NK cell (NK1.1) antibodies followed by depletion with dynabeads sheep anti-rat IgG (Invitrogen, Carlsbad, CA, USA).

2.4. $CD4^+$ T cell priming by the DC subsets in vitro

Naive OVA-specific $CD4^+$ T (3×10^5) cells purified from OT-II mice were co-cultured either with sorted $CD8\alpha^+$ or $CD8\alpha^-$ DCs (each 0.3×10^5 DCs, 1:10 ratio of DC to T cells) per well in the round bottom 96 well plate for 3 days in the presence of 25 μg/ml poly (I:C) or 100 ng/ml LPS with 2 μM OVA peptide (a.a. 323–339) (Genscript, Piscataway, NJ, USA). Then, cytokines in supernatants were detected by ELISA.

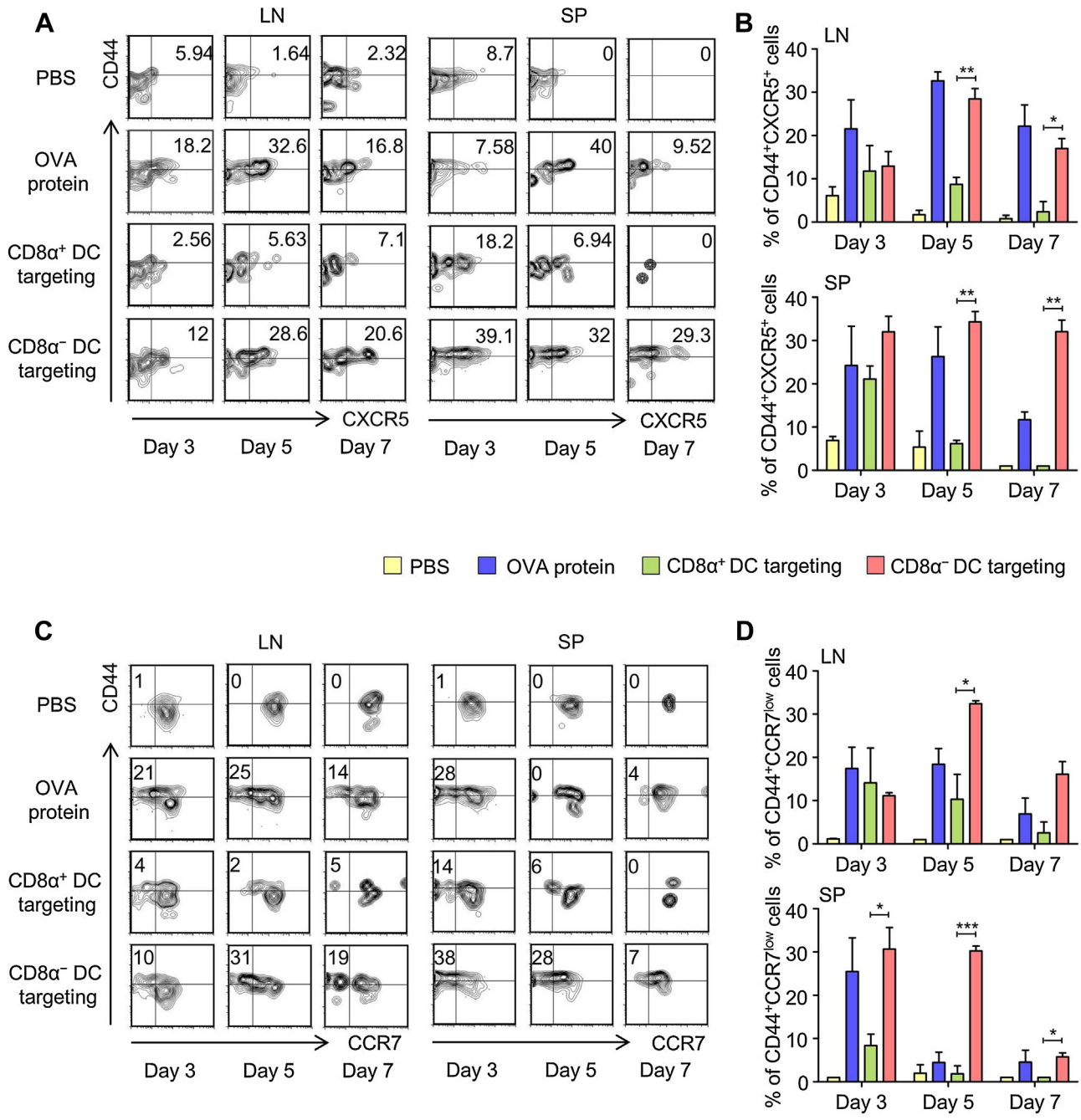

Fig. 2. Induction of CXCR5$^+$CCR7^{low} Tfh cells by CD8α$^-$ DCs *in vivo*. (A–D) OVA specific DO11.10$^+$Thy1.1$^+$CD4$^+$ T cells were adoptively transferred to naive Thy1.2$^+$ BALB/c mice at day −1, and were immunized s.c. with PBS, soluble OVA protein (OVA protein), or either αDEC:OVA (CD8α$^+$ DC targeting) or αDCIR2:OVA (CD8α$^-$ DC targeting) conjugated mAbs in the presence of poly (I:C) at day 0. At each indicated time point after the immunization, lymph node (LN) or spleen (SP) cells were prepared and Tfh cells gated from the Thy1.1$^+$CD4$^+$ T cells were analyzed. (A and C) Representative flow cytometry plots of CD44$^+$CXCR5$^+$ (A) or CD44$^+$CCR7^{low} (C) Tfh cells. (B and D) Data represent mean ± s.e.m. of three independent experiments described in (A) or (C), respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (A–D, $n = 3$ per group).

2.5. *Induction of CD4$^+$ T cells by DC subset targeting* in vivo

Isolated CD4$^+$ T cells from DO11.10 Thy 1.1 mice were adoptively transferred (3×10^6 cells per mouse) into naïve Thy 1.2$^+$ BALB/c mice intravenously (i.v.) at day −1. At day 0, PBS, 500 μg soluble OVA protein (endotoxin-free; Seikagaku Corp, Tokyo, Japan), or 5 μg of each distinct DC subset targeting monoclonal antibodies (anti-DEC-205 or anti-DCIR2 mAbs) genetically conjugated with OVA protein in the presence of 50 μg poly (I:C) were injected via the footpads of the mice. At the indicated time points after the immunization, single cells from the lymph nodes or the spleen were prepared and analyzed for the expression of various molecules by flow cytometry. In some experiments, 5 μg of each distinct DC subset targeting mAbs conjugated with OVA protein + 50 μg soluble OVA protein with 50 μg LPS were injected via the footpads of the mice at day 0.

2.6. *Production of DC subset-specific targeting antibodies conjugated with OVA protein*

DC subset-specific targeting antibodies conjugated with OVA protein were used as previously described [11,12]. All proteins used were free of endotoxin (<0.125 endotoxin units/mg) in a Limulus

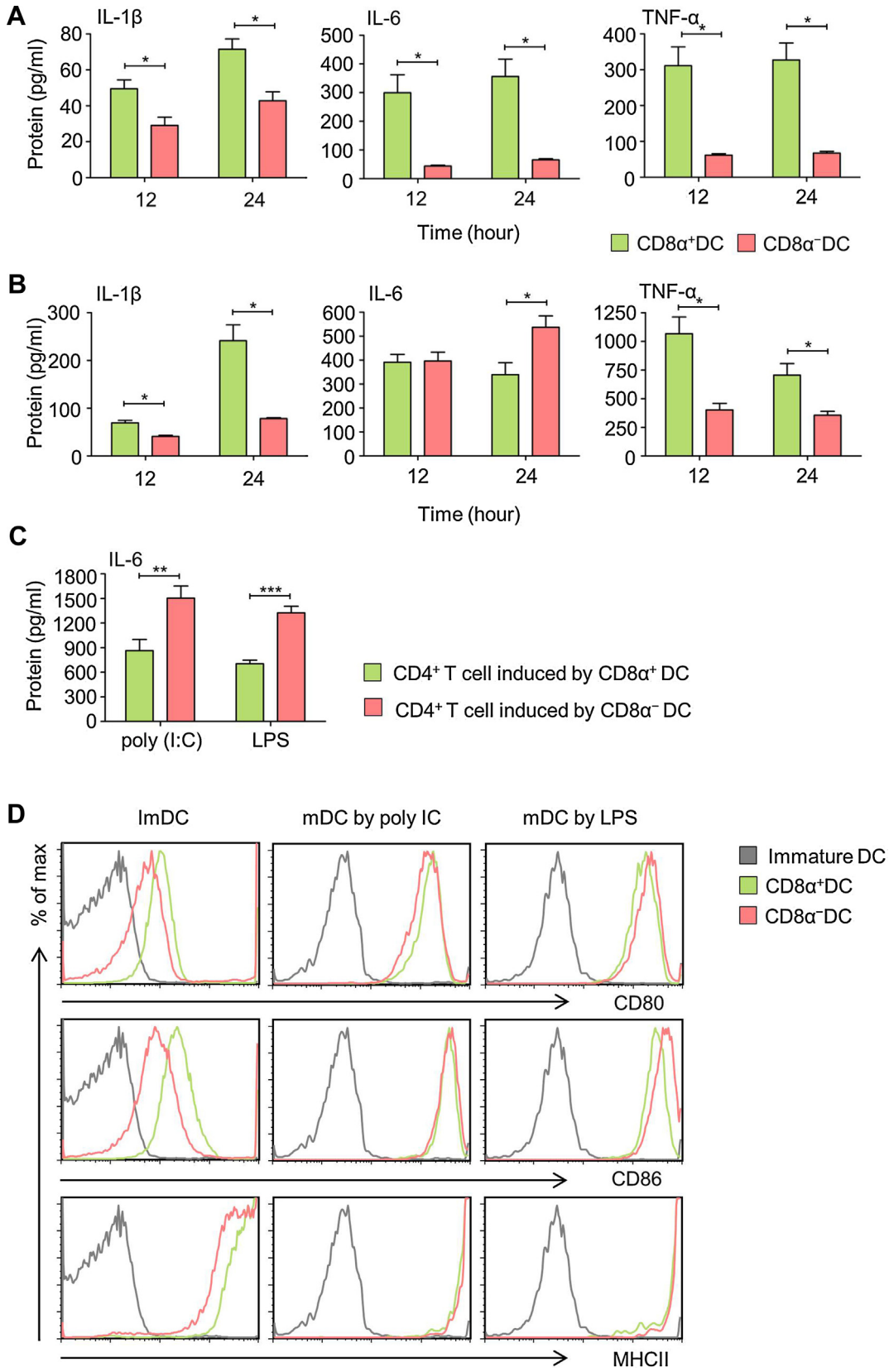

Fig. 3. IL-6 cytokine secreted from the DC subsets or the CD4+ T cells induced by the DC subsets and maturation markers on the DC subsets. (A and B) ELISA analysis of the cytokines, IL-1β, IL-6, TNF-α, secreted from each purified DC subset stimulated either with poly (I:C) (A) or LPS (B) for 12 or 24 h. * $P < 0.05$. Data represent mean ± s.e.m. of three independent experiments (A and B). (C) OT-II OVA specific Vα2+CD4+ T cells were co-cultured with each DC subset in the presence of OVA peptide (323–339) either with poly (I:C) or LPS. After 3 days of the co-culture, the level of IL-6 cytokine in the supernatant was measured by ELISA. ** $P < 0.01$, *** $P < 0.001$. (D) Maturation markers expressed on the two DC subsets. Each DC subset was stimulated either with poly (I:C) or LPS for 0 (immature) or 24 h (mature). Representative histograms of CD80, CD86 or MHCII expressed on the two DC subsets. Data are representative of three independent experiments.

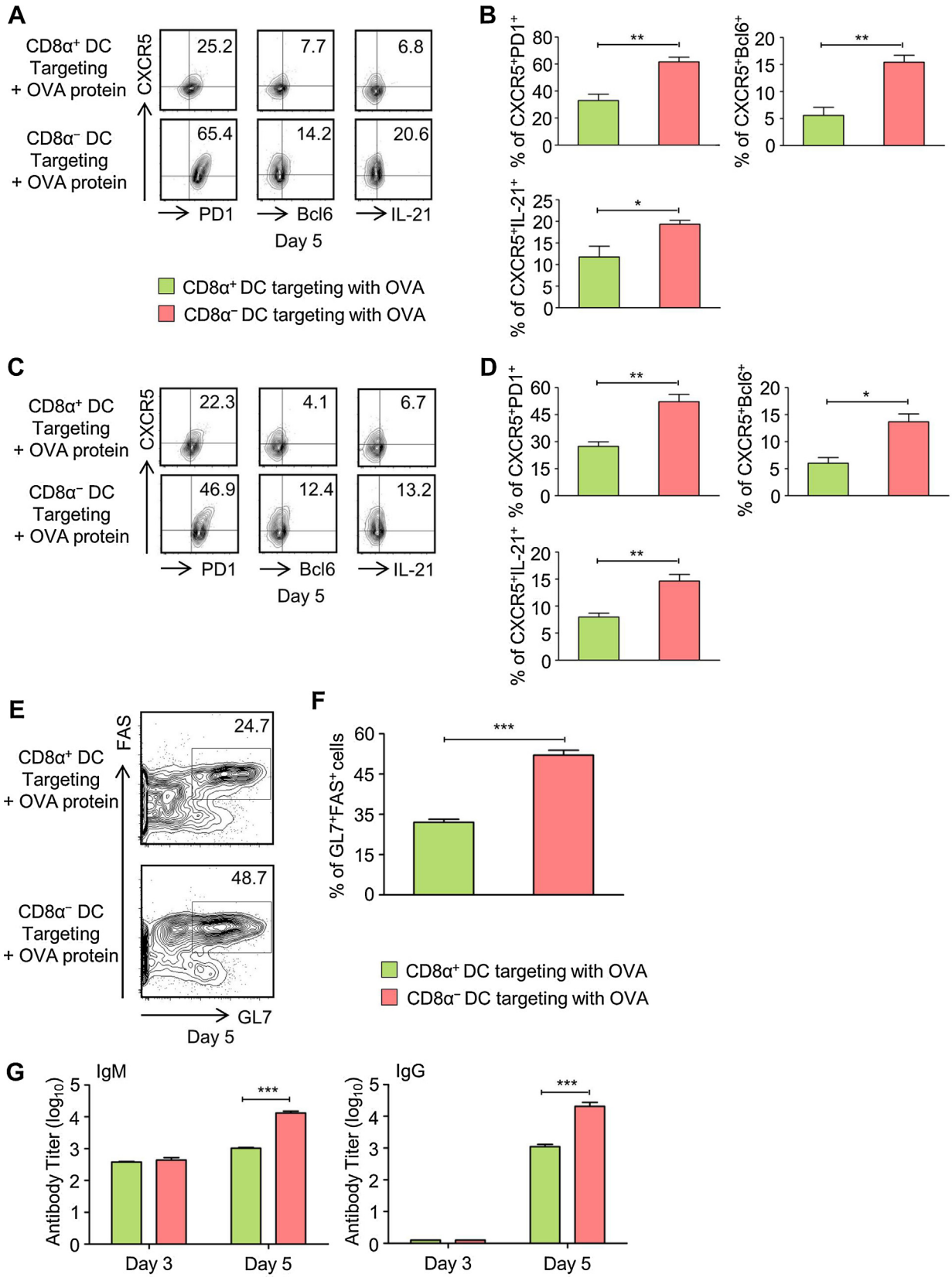

Fig. 4. CD8α⁻ DCs specialize in inducing functional Tfh cells *in vivo*. OVA specific DO11.10⁺CD4⁺ T cells were adoptively transferred to naive BALB/c mice at day −1, and were immunized s.c. either with αDEC:OVA (CD8α⁺ DC targeting) or αDCIR2:OVA (CD8α⁻ DC targeting) conjugated mAbs in the presence of soluble OVA protein + LPS. 5 days after the immunization, lymph node cells (A and B) or spleen cells (C–F) were prepared and Tfh cells gated from the DO11.10⁺CD4⁺CD44⁺ T cells were analyzed. (A and C) Representative flow cytometry plots of CXCR5⁺PD1⁺, CXCR5⁺Bcl6⁺, or CXCR5⁺IL-21⁺ Tfh cells. (B and D) Data represent mean ± s.e.m. of three independent experiments described in (A) or (C), respectively. (E) Representative flow cytometry plots of GL7⁺FAS⁺ germinal center (GC) B cells gated from CD19⁺IgD⁻ splenocytes. (F) Data represent mean ± s.e.m. of three independent experiments described in (E). (G) ELISA analyses of OVA-specific serum IgM or IgG antibodies. Data represent mean ± s.e.m. of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (A–G, $n = 3$ per group).

Amebocyte Lysate assay, QCL-1000 (Bio Whittaker, Chesterbrook, PA, USA).

2.7. ELISA for serum antibodies and cytokines

High binding ELISA plates (BD Bioscience, San Jose, CA, USA) were coated with 10 μg/ml OVA protein overnight at 4 °C. Plates were washed 3 times with PBST (PBS with 0.1% of Tween 20) and blocked with PBST-BSA 5% for 1 h at 37 °C. Serial dilutions of mouse serum were added and incubated for 1 h at 37 °C. Secondary goat anti-mouse IgM or IgG specific antibodies conjugated with horseradish peroxidase were added and incubated for 1 h at 37 °C, and then developed with *o*-phenylenediamine (Sigma–Aldrich, St. Louis, MO, USA) tablets at room temperature for 15 min. Data are presented as log antibody titers, with the highest dilution showing OD_{450} >0.1. To detect cytokines in supernatants, we used CBA flex sets (BD Bioscience) and the concentrations of various cytokines were measured by flow cytometry.

2.8. Immunohistochemistry

Briefly, tissue sections (10 μm) of the spleen were prepared by using Cryotome (Thermo Fisher scientific, Waltham, MA, USA) and then fixed with aceton on a slide. For localization images of DCs and $CD4^+$ T cells, samples were stained with BV-421 anti-mouse IgD (BioLegend, San Diego, CA, USA), PE anti-mouse DO11.10 (BD Biosciences), and APC anti-mouse DEC205 or DCIR2 (BioLegend). Then, the stained samples were imaged with FV10i (Olympus, Southend-on-Sea, UK) confocal microscope.

2.9. Flow cytometry

Data were acquired by BD FACS Calibur or BD LSR fortessa and analyzed by FlowJo software (TreeStar, Ashland, OR, USA), shown on the log scale graph (from 10^1 to 10^4 or 10^5). All bar graph data values were shown after subtracting their corresponding isotype values. Median Fluorescence Intensity was abbreviated as MFI. Used antibodies in flow cytometry as follows: CD11c (HL3), CD8 (53-6.7), CD3 (145-2C11), B220 (RA3-6B2), Vα2 (B20.1), Thy1.1 (OX-7), DO11.10 (KJ1-26), CD4 (RM4-5), CD44 (IM7), CXCR5 (2G8), Bcl6 (K112-91), CCR7 (4B12), CD19 (1D3), IgD (11-26C.2A), FAS (Jo2), GL7 (GL7), CD80 (16-10A1), CD86(GL1), MHCII (M5/114), Rat IgG2a, κ (R35-95), and Rat IgG2b, κ (A95-1) were purchased from BD biosciences; PD1 (RMP1-30) and IL-21 (mhalx21) were purchased from eBioscience, San Diego, CA, USA.

2.10. Intracellular staining

Cells were stained with anti-mouse DO11.10, CD4, CD44 and CXCR5 for 30 min at room temperature. Following fixation and permeabilization with Cytofix/Cytoperm kit (BD Biosciences) according to the manufacturer's protocol, the cells were stained for intracellular Bcl6 or IL-21 along with their isotypes for 25 min at 4 °C. Data were analyzed by flow cytometry.

2.11. Statistics

Results are expressed as mean ± s.e.m. We used PRISM 4.0 program (GraphPad Prism, La Jolla, CA, USA) and performed non-parametric Mann–Whitney U test when appropriate. The *P* values <0.05 were considered significant.

3. Results

3.1. $CD8\alpha^-$ DC induced $CD4^+$ T cells are localized proximal to $CD8\alpha^-$ DCs in the marginal zone (MZ) bridging channels

In order to deliver ovalbumin (OVA) antigens to each DC subset specifically *in vivo*, we utilized DC subset targeting monoclonal antibodies (mAbs) genetically conjugated with OVA protein, αDEC-205:OVA (for targeting $CD8\alpha^+$ DCs) or αDCIR2:OVA (for targeting $CD8\alpha^-$ DCs) mAbs. Then, we examined the localizations of the two DC subsets together with OVA antigen specific $DO11.10^+CD4^+$ T cells primed by each of the DC subsets. As previously reported [9], we confirmed the different localizations of the two DC subsets in the spleen; $CD8\alpha^+$ DCs predominantly localize in the T cell enriched region while $CD8\alpha^-$ DCs localize in the MZ bridging channels (Fig. 1). Interestingly, the $CD4^+$ T cells induced by $CD8\alpha^-$ DCs tended to localize in the MZ bridging channels proximal to $CD8\alpha^-$ DCs as early as 1 day after the immunization, and 3 and 5 days after the immunization, the $CD4^+$ T cells predominantly localized in the MZ bridging channels. In contrast, the $CD4^+$ T cells induced by $CD8\alpha^+$ DCs remained in the T cell enriched region at all-time points tested (Fig. 1).

3.2. $CD4^+$ T cells induced by $CD8\alpha^-$ DCs express high levels of CXCR5 and low levels of CCR7

Based on the fact that pre-Tfh cells up-regulate CXCR5 while down-regulating CCR7 to migrate into the inter-follicular region/MZ bridging channels [13,14], where the development of Tfh cell initiates [15], we examined the expressions of CXCR5 and CCR7 on the antigen specifically activated $CD44^+CD4^+$ T cells induced by the two DC subsets. We found high expression of CXCR5 on the $CD4^+$ T cells induced by $CD8\alpha^-$ DCs compared with that on the $CD4^+$ T cells induced by $CD8\alpha^+$ DCs at 5 and 7 days after the immunization, both in the lymph node and the spleen (Fig. 2A and B), and the percentage of $CCR7^{low}CD4^+$ T cells induced by $CD8\alpha^-$ DCs was significantly increased (Fig. 2C and D), suggesting that the pre-Tfh cells induced by $CD8\alpha^-$ DCs tended to localize in the MZ bridging channels via CXCR5–CXCL13 interaction. In contrast, $CD8\alpha^+$ DC induced $CXCR5^{low}CCR7^{high}$ $CD4^+$ T cells remained in the T cell enriched area via CCR7-CCL19/21 interaction (Fig. 2A–D).

3.3. Level of IL-6 cytokine secreted from the two DC subsets is dependent on an adjuvant type

Despite previous reports demonstrating the importance of interleukin-6 (IL-6) in inducing Tfh cells [16,17], its source, together with the possibility of its secretion from the DC subsets, were not clearly understood. In order to examine and quantify IL-6 and other cytokines secreted from each DC subset, we isolated the two DC subsets, $CD3^-B220^-CD11c^+CD8\alpha^+$ DCs and $CD3^-B220^-CD11c^+CD8\alpha^-$ DCs. Each purified DC subset was stimulated either solely with poly (I:C) or LPS for 12, 24 h *in vitro*. Unexpectedly, upon poly (I:C) stimulation, we found significantly enhanced IL-6 secretion from $CD8\alpha^+$ DCs compared with that from $CD8\alpha^-$ DCs (Fig. 3A). However, in the presence of LPS, IL-6 secretion amount was similar between the two DC subsets 12 h after the stimulation and its level from $CD8\alpha^-$ DCs became higher 24 h after the stimulation (Fig. 3B). Interestingly, the OVA specific $CD4^+$ T cells induced by $CD8\alpha^-$ DCs in the presence of OVA peptide secreted significantly higher level of IL-6 cytokine regardless of the types of adjuvant (Fig. 3C), suggesting the lesser role of IL-6 secreted from the two DC subsets in inducing Tfh cells. Pro-inflammatory cytokines such as IL-1β and TNF-α were highly secreted from $CD8\alpha^+$ DCs both upon poly (I:C) and LPS stimulations (Fig. 3A and

B). The expression levels of maturation markers CD80, CD86 and MHCII were comparable between the two DC subsets (Fig. 3D).

3.4. CD8α⁻ DCs are superior in inducing functional Tfh cells

One previous study demonstrated that CD8α⁻ DCIR2⁺ DCs have a unique capacity to initiate extra-follicular B cell responses through rapid activation of Ag-specific B cells [18], suggesting the possibility that the two DC subsets are differentially able to deliver antigens to cognate B cells in inducing antigen specific humoral immune responses. In order to examine the effect of different antigen accessibility of APCs including B cells on the induction of Tfh cells upon distinct DC subset targeting, we designed an experiment to equivalently provide antigens to all APCs in both CD8α⁺ and CD8α⁻ DC targeted groups. When we immunized mice with 5 μg of each DC subset specific targeting mAbs conjugated with OVA protein (each immunization contains ~1.6 μg OVA protein), we also simultaneously injected 50 μg soluble OVA protein to the mice to ensure that B cells and other APCs have equivalent access to the antigen. Five days after the immunization, we observed significantly higher percentages of CXCR5⁺PD1⁺, CXCR5⁺Bcl6⁺ and CXCR5⁺IL-21⁺ Tfh cells both in the lymph nodes (Fig. 4A and B) and the spleen (Fig. 4C and D) from the CD8α⁻ DC targeted group compared with those from the CD8α⁺ DC targeted group. In addition, the percentage of CD19⁺IgD⁻GL7⁺FAS⁺ GC B cells was higher in splenocytes from the CD8α⁻ DC targeted group (Fig. 4E and F). The titers of OVA antigen specific IgM were observed to be comparable between CD8α⁺ and CD8α⁻ DC targeted groups at day 3. At day 5, OVA antigen specific IgM or IgG titers were higher in the CD8α⁻ DC targeted group compared with those in CD8α⁺ DCs (Fig. 4G).

4. Discussion

Modulating Tfh cell dependent humoral immune responses is the key step to regulate defects in humoral immunity. However, the induction of Tfh cell differentiation by DCs has not been clearly understood. We here investigated the undescribed intrinsic features of the two conventional DC subsets important for the initiation of Tfh cell differentiation.

It has been reported that the localization of DCIR2⁺CD4⁺ DCs (closely equivalent to DCIR2⁺CD8α⁻ DCs in this study) in the MZ bridging channel/inter-follicular region is mediated by the chemotactic receptor EBI2 [19,20], having an important role in inducing blood-borne particulate antigen specific CD4⁺ T cell and B cell immune responses. Supported by these previous studies, we suggest that the localization of DCIR2⁺CD8α⁻ DCs may provide advantages in Tfh cell induction since we observed the recruitment of antigen specific CD4⁺ T cells induced by CD8α⁻ DCs into MZ bridging channels as early as 1 day after the stimulation. In addition, these CD4⁺ T cells localized in the MZ bridging channels were presumably mediated by the high level of CXCR5 and the low level of CCR7 expressions consistent with the previous studies [13,14]. The CD4⁺ T cells induced by CD8α⁺ DCs expressed low level of CXCR5 and high level of CCR7 which may maintain their localization in the T cell enriched region and result in the differentiation to IFN-γ secreting Th1 cells [10].

The fate of various CD4⁺ T cell subsets is often determined by the secretory cytokines [21], and IL-6 has been noted as a key cytokine to induce Tfh cell differentiation [22]. Our previous study showed that the functional Tfh cells were well induced by CD8α⁻ DCs both in the presence of poly (I:C) and LPS [10]. However, the two DC subsets displayed opposites in IL-6 secretion in the presence of poly (I:C) and LPS, suggesting the insignificance of DC-secreted IL-6 in the induction of Tfh cells. Instead, the high levels of autocrine IL-6 (Fig. 3C) secreted from the antigen specifically activated CD4⁺ T cells induced by CD8α⁻ DCs, independent of the adjuvant type, seem to be critical for Tfh cell induction. The pro-inflammatory cytokines such as IL-1β and TNF-α, highly secreted from CD8α⁺ DCs, may have a role in inducing other CD4⁺ T cell subsets [21].

Previous studies reported the advantageous localization of CD8α⁻ DCs in MZ bridging channels to encounter antigen specific B cells [18] as well as their slow rate of antigen internalization for prolonged antigen presentation [9], which may provide more chances for B cells to uptake antigens, resulting in the highly activated antigen specific B cells. However, we confirmed the superiority of CD8α⁻ DCs over other APCs in the initiation of functional Tfh cell induction in the presence of excess OVA antigens. Nevertheless, since the deficiency of functional B cells resulted in the decreased number of Tfh cells [14,23], and since other DC subsets such as late activator antigen-presenting cells [24], monocyte-derived DCs [25], Langerhans cells [26] or CD103⁺dermal DCs [27] promote functional Tfh cell differentiation upon different types of immune responses, further researches are required to understand how APCs interplay each other in the induction process of Tfh cells. In addition, given the previous reports demonstrating the involvement of B cells and their products, the immunoglobulins in modulating DC functions to induce distinct T cell subsets such as Treg or Th2 cells [28,29], the role of B cells in CD8α⁻ DC induced Tfh cells needs to be investigated.

Together with ICOSL and OX40L expressed on CD8α⁻ DCs in inducing Tfh cells [10], supported by the previous studies demonstrating the critical role of ICOSL and OX40L on dendritic cells or APCs in inducing Tfh cells [30,31], the localization of CD8α⁻ DCs in MZ bridging channels may provide synergic effects in the initiation of Tfh cell differentiation. Other factors in DC subsets that may need to be considered for Tfh cell differentiation are the difference in antigen processing [9] and the duration of antigen presentation [32,33].

In conclusion, we show the previously undescribed intrinsic features of CD8α⁻ DCs, suggesting its specialty in inducing functional Tfh cells. Given the results we demonstrated so far, we provide a rationale to find the counterpart of CD8α⁻ DCs in humans to develop efficient DC-based vaccines for enhanced humoral immune responses.

Author contributions

C.S., J.H., Y.D. and S.R. conceived and designed the experiments. C.S., J.H. and B.C. performed the experiments. C.S., J.H., Y.D. and S.R. analyzed the data. C.S., J.H., Y.C. and S.R. wrote the manuscript. All authors contributed to critically revise the manuscript for important intellectual content, and gave final approval and agree to be accountable for all aspects of the work.

Conflict of interest

Authors have no commercial or financial conflict of interest.

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